# Increased Runx-2 Expression In The Y-TZP Biomaterial Osteointegration Process With hADMSC Seeding

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**KEYWORDS**
Runx-2 Expression, Hadmsc, Osteointegration, Biomaterial, Y-TZP

**ABSTRACT**
Introduction:. Peri-implantitis, overload, and poor prosthetic construction result in dental implant failure. The success factor of the implant is osteointegration, the formation of tissue between the implant surface and the bone without fibrous tissue. Human Mesenchymal bone marrow (hBMSC) is difficult and risky. Biomarkers associated with increased osteogenesis for osseointegration of biomaterials. This research was conducted to analyze biomarkers that can occur osteogenesis, to increase osteointegration of biomaterials that have high biocompatibility using mesenchymal stem cells. Methods: The research carried out is a true experimental study on Wistar rat test animals totaling 6 treatment groups consisting of 3 groups at the 1st week and 8th week. Each tissue sample was made into a slice preparation with a thickness of 4um, then detected by immunohistochemical examination for Runx2 expression and data in analysis. Results: An increase in Runx-2 expression occurred significantly at week 8 in the Y-TZP biomaterial treatment group seeded with hADMSC (P4). Disscusion: Through increased expression of Runx-2 is a biomarker as a marker on hADMSC which can increase osteogenesis and osteointegration of Y-TZP biomaterial. The use of Y-TZP biomaterial in tissue engineering can be used in other fields of medicine, due to its good biocompatibility as an implant biomaterial so that implant failure can be prevented. Conclusion: Dental implant failure prevented by hADMSC seeding in Y-TZP via increased Runx-2 extrusion. Runx-2 in osteogenesis and fibroblast migration are influenced by various genes in the bone healing process. The benefits of this research can reduce the stiffness of dental implants.

## 1. Introduction

Tooth loss requires the replacement of a fixed prosthesis, namely a dental implant made of titanium. There are significant differences in one of the main indicators of oral health is tooth loss in a population and between populations (1). There is a shrinkage of the dimensions (height and width) of the alvelar ridge which depends on several aspects, so the physiological remodeling process on hard and soft tissues occurs after tooth loss (2). There are two causes as reasons for tooth loss: periodontal (tooth extraction or loss due to advanced periodontitis or combined periodontal-endodontic lesions) and nonperiodontal (crown/root remains; endodontic and periapical lesions; fractures of bones and crowns; trauma; or other clinical causes/ tooth agenesis) (3). Stage IV periodontitis is the cause of tooth loss so rehabilitation with implants is the gold standard (4). Psychological trauma can be caused by tooth loss that requires a treatment modality with dental implants that replace the missing tooth (5). Dental implants can rehabilitate bones with poor quality conditions and difficult torque.(6)

Dental implant biomaterials must have high biocompatibility. Peri-implantitis, overload, and poor prosthetic construction result in dental implant failure (7). Effective soft tissue sealing around the implant can protect the alveolar bone from bacterial invasion, which is critical for the optimal long-term function of dental implants(8). Micro-rough structure can be applied to new dental implant biomaterials(9). Dental implants occur mechanical and biochemical bonds that integrate into the bone occur through a layer of protein and glycoprotein formed at the bone-dental implant interface (10). There are limitations in the biocompatibility of titanium dental implants that cannot guarantee interaction with bone and soft tissue(11). Dental implantation has been used to restore lost teeth The success is due in large part to osseointegration (12) Osteointegration of titanium dental implants is related to surface roughness which is related to the inflammatory response in the peri-implant tissue and dental implant particles(13).

The osteointegration process is one of the successes in dental implant installation. The success factor of the implant is osteointegration, the formation of tissue between the implant surface and the bone without fibrous tissue (14)(15). The metabolic and remodeling process between the bone tissue and the implant surface makes for increased osteointegration (16). Functional and aesthetic outcomes occur due to the osteointegration of the implant Polarization of macrophages as the effect of implant materials determines the osteointegration effect and is more stable (17). The occurrence of complications includes incorrect prosthesis, peri-implanitis

mucositis so that the implant can be lost because the osteointegration process has not been completed (18)(19). The topography of the titanium surface during dental implant insertion is a success is osteointegration (20)(21)(22)(23). The active process in osteointegration at the histological level is the insertion of the implant and its maintenance (24). Prevention and management of peri-implant lesions in the early stages by using bioactive in osteointegration of dental implants (25)(26).

The most potent mesenchymal stem cells (MSC) are those from bone marrow which can increase ostegenesis. Bone fractures, infections, tumors, trauma and osteotomy reduce the mechanical strength of bones and interfere with the biological function of bones called bone defects (27). Bone repair occurs an interaction between mesenchymal stem cells, inflammatory cells and angiogenic cells (28). MSC therapy is a group of cells that can renew themselves and have the potential to differentiate to accelerate bone healing (29). Human bone marrow mesenchymal stem cell (hBMSC) is a strong, immunosuppressive, and multi-lineage differentiated stromal cell (30). The precursor cells of osteoblasts that can overcome osteoporosis are hBMSC (31). The ability of long non-coding RNA (IncRNA) to modulate the differentiation of MSCs in the formation of new bone i.e. hBMSC (32). Regulation of decreased miR-137 expression occurs during osterogenic differentiation of hBMSC (33).

Osteogenesis improves the osteointegration of dental implant biomaterials. Osteogenesis occurs in Ti6AI4V/HA composites as dental implants (34). Osteogenesis (formation of bone calluses) and bone stability occur postoperatively (35). Addition of hBMSCs on Ti surfaces (cell adhesion, proliferation, osteogenic differentiation, and gene expression associated with osteogenesis) (36). Implant fixation biologically forms new bone called osteogenesis (37). Accelerate bone repair and peri-implanted bone formation and improve bone contact ratio, bone volume fraction, trabecular number and thickness by evaluating osteogenesis and osteointegration (38). The ability of osteogenesis and antibacterial activation is a strategy in dental implants showing the biofunctionalization of the implant layer (39).

Dental implant failure is due to the rejection of biomaterial The inflammatory response to the pathogen is peri-implanitis is the main cause of dental implant failure due to the inability to heal soft tissues due to infection, inflammation, and function of keratinocytes and macrophages. (40). Etiology and pathogenesis of peri-implanitis causes of dental implant failure (41). Local bacterial infections that cause dental implant failure by modifying texture and roughness or coating with antibiotics (42). Loss or removal of implants for any reason is called dental implant failure (43). Dental implant failure due to the use of other drugs (nonsteroidal anti-inflammatory drugs and selective serotonin reuptake inhibitors) (44).

Allergic factors can cause the rejection of biomaterials. Wound severity, allergic reactions, hematomas, seromas, implant wobbly, reoperation, and bone-implant fusion status are clinical outcomes as side effects (45). biomaterial corrosion, implant wobbling, and biological factors (allergic reactions and inflammation) that cause rejection of the implant material (46). Accumulation of macrophages and -T lymphocytes and a decrease in B-lymphocytes indicate Type 4 (47). Hypersensitivity as an allergic reaction to titanium dental implants (48). Degradation of implant materials, allergic reactions, and chronic peri-implant inflammation as biomechanical and biological related to implant failure and loss (49).

There are often failures in the installation of dental implants because there is no osteointegration. The insufficient osteointegration between titanium (Ti) based dental implants and surrounding bone tissue under osteoporotic conditions, as well as implant-associated infections usually associated with dental implant failure (50). Several attempts have been made to improve the bonding between bone and a dental implant by coating the titanium of the implant with bioactive materials, like hydroxyapatite, which can form a chemical bond with bone tissue. Despite the development of this coating method, implants have continued to fail. An exposed implant can interact with negative bacteria, which can lead to infection around the implant (47). P. aeruginosa, a major respiratory pathogen, is a gram-negative, aerobic/facultative anaerobe, rod-shaped bacterium. It has been isolated from peri-implant diseased sites and is associated with dental implant failure (51). it seems that smoking, early infection, fresh socket placement, and placement of implants along with bone substitutes may increase the failure rate after acute infection in dental implant placement(52). Allelic variation in cytokine genes and factors regulating their expressions result in phenotypic differences in cytokine responses among individuals, which may be important in susceptibility and progression of diseases such as periodontal disease and dental implant failure(53).

Human Mesenchymal bone marrow (hBMSC) is difficult and risky. hBMSC is an important component of the bone marrow microenvironment (54) hBMSC has increased proliferation, adhesion, matrix-receptor interactions.(55). hBMSC is also a non-hematopoietic multipotent cell that can be differentiated into mesodermal cells (osteoblasts and adipocytes)(56). Monitoring of alkaline pospatase (ALP) activity can be evaluated through the differentiation of hBMSC into bone tissue (57). decreased osteogenic expression of transcription factor (Runx-2) and osteopontin (OPN) in hBMSC knockdown USP7 as regenerative medicine and tissue engineering (58). Side effects occurred in the hBMSC intervention, namely: allergies, hypotension, anaphylaxis, bronchospasm, lung cell embolism.(59). Multiple miRNAs regulate a single mRNA that can improve hBMSC differentiation (60).

Many biomarkers are not necessarily directly related to increasing osteogenesis to osseointegrate biomaterials. Promotion of the P38 signaling pathway via IL-32 to a therapeutic target or biomarker in bone formation mechanisms by hUMSC(61). Measurement with biomarkers to analyze osteoblasts and osteoclasts in bone formation in increased trabecular bone mass(62). Overexpression of mir-381-3p occurs through differentiation through the activity and mineralization of ALP as a biomarker(63). In bone formation are related several signaling pathways, growth factors, cytokines and other components (64). During osteogeneic division cells into expression of ALP+ and ALP fractions and changes in CD26, CD34 and CD271 after differentiation (65).

This research was conducted to analyze biomarkers that can occur osteogenesis, to increase osteointegration of biomaterials that have high biocompatibility using mesenchymal stem cells. The use of hADMSC with Y-TZP biomaterial is increase in the acceleration of osteointegration by analyzing the Runx-2 biomarker. This study aims to improve the osteointegration of Y-TZP biomaterials seeded by hADMSC by analyzing the Runx-2 biomarker.

## 2. Methods

The research carried out is a true experimental study on Wistar rat test animals totaling 6 treatment groups consisting of 3 groups at the 1st week (Positive control, defects with Y-TZP, and defects with Y-TZP seeding hADMSC) and 3 groups at the 8th week (Positive control, defects with Y-TZP, and defects with Y-TZP seeding hADMSC), as well as 1 negative control group, Each group consisted of 4 samples, with a Randomized Post Test Only Control Group Design. The histopathological examination determined the expression of Runx-2 on the mandibular bone surface of rats of Rattus norvegicus albinus wistar strain after Y-TZP scaffold implantation for 1 week and 8 weeks. Each tissue sample was made into a slice preparation with a thickness of 4um, then detected by immunohistochemical examination for Runx2 expression. Data analysis was done by comparing the immunohistochemical (IHC) expression results from Runx-2.

## 3. Result and Discussion

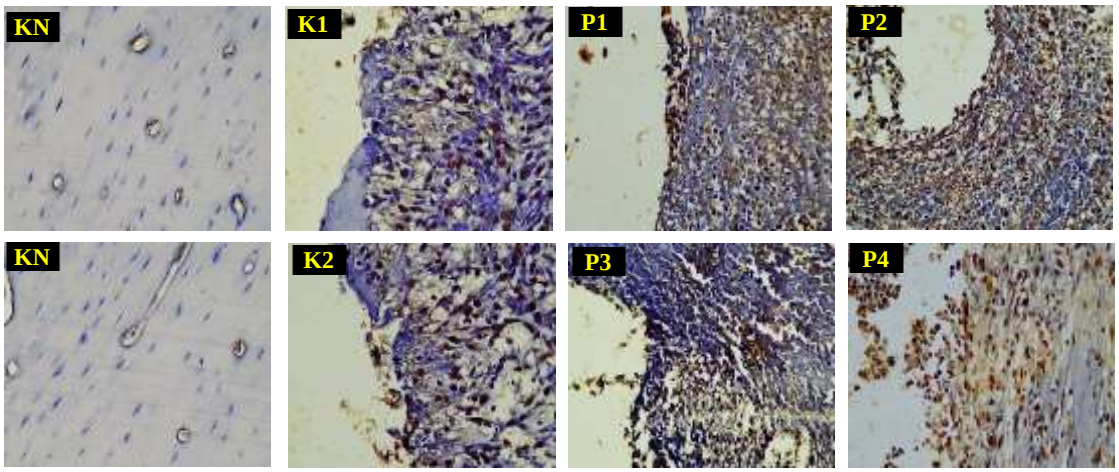


Figure. 1 Runx2 expression at weeks 1 and 8 of the treatment provided a brownish reaction to the *anti-monoclonal anti-Runx2* using a *Nikon Eclipse Ci camera, 16.25 megapixel DS-Ri camera*, 400x magnification

The expression of Runx2 in each treatment showed a brownish reaction to anti-monoclonal anti-Runx2 which can be seen in the figure. 1.

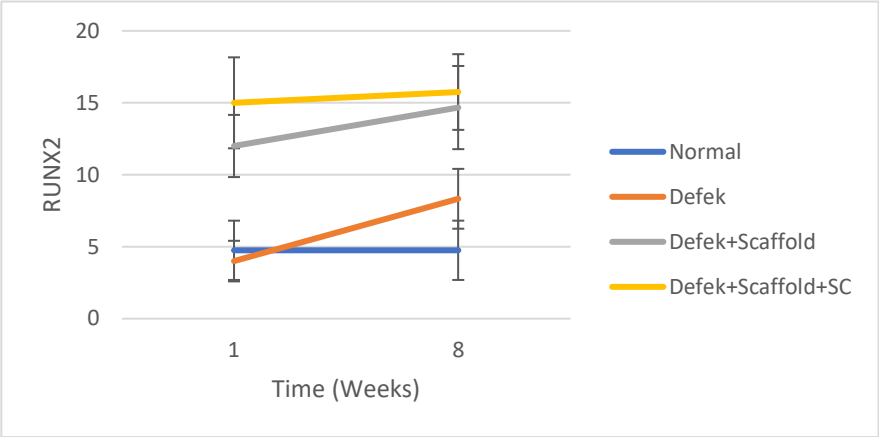


Figure. 2 Intergroup Runx-2 diagram

The results of the Runx2 difference test between groups after the normality test was not distributed normally, then tested with the Kruskal-Wallis significant test $p<0.05$. The Wilcoxon-Mann Whitney test found that K1 and K2 were no difference between groups with KN, while P1, P2, P3, and P4 were different between groups with KN, K1, and K2 shown in the figure. 2. An increase in Runx-2 expression occurred significantly at week 8 in the Y-TZP biomaterial treatment group seeded with hADMSC (P4).

Through increased expression of Runx-2 is a biomarker as a marker on hADMSC which can increase osteogenesis and osteointegration of Y-TZP biomaterial. Runt-related transcription factor 2 (Runx-2) as one of the specific transcription factors in bone through osteoblast differentiation determined by MSC. At the end of the differentiation of osteoblasts, the secretion of matrix proteins occurs to replenish the bone matrix (type 1 colagen/Col-1, Osteocalcin/OCN, Alkaline phosphatase/ALP)(66). The increase in osteogenesis by MSCs is due to increased expression of Runx-2 and other osteogenesis-related genes. MSCs differentiate towards osteoprogenitor and proosteoblasts directly or indirectly regulating the number of other specific genes for type 1 collagen and osterix (Osx) with increased expression of Runx-2 (67).

The increase in bone matrix protein gene expression occurs due to positive regulation of Runx-2 as a marker of osteogenesis at the DNA level which will increase the number of osteoblast cells that inhibit bone reproduction due to tooth extraction inflammation (48). Regulation of bone formation and modulation of expression of other genes involved in osteoblast differentiation processes is an important role of Runx-2 expression in a broader spectrum in bone formation (cell growth patterns and proliferation activity) (68). Runx-2 and ALP in osteogenesis and fibroblast migration are influenced by various genes in the bone healing process (48). An increase in Runx2 in preosteoblasts can affect an increase in ALP which can increase osteoblasts, but an increase in the number of osteoblasts does not significantly increase bone density because Runx2 has not decreased at 8 weeks. Mesenchymal as a positive precursor of osteoblasts, namely Runx2 switched to osteoblast progenitors, Runx2 and Osx paired before adopting the phenotype of adult osteoblasts. The positive precursor of osteoblasts, Runx2, increased and decreased Osx in preosteoblasts, after which there was a decrease in Runx2 and an increase in Osx in osteoblasts.

MSC with the same potency as hBMSC is hADMSC found in the source of fat tissue analyzed with increased expression of Runx-2. hADMSC has better protective effects than hBMSC and hUCMSC(69). Through the NF-κB pathway as an inflammatory marker there are protective, proliferative antioxidant and anti-inflammatory effects on hADMSC isolation (70). Applications in the Regenerative field of medicine of the future are in hADMSC (71). Applications in the Regenerative field of medicine of the future are in hADMSC (72). immunomodulation and effect of increasing tissue regeneration on hADMSC (73). hADMSCs seeded on Y-TZP biomaterial help in the osteoingation process avoiding dental implant failure.

Dental implant failure can be overcome by administering hADMSC to improve osteointegration with increased osteoblasts in the osteogenesis process. Early dental implant failure is defined as the loss or removal of an implant for any reason loss or removal of implants due to any reason (43). The main cause of dental

implant failure is peri-implanitis (41). The inflammatory response of the pathogen causes peri-implanitis (40). The release of ions and the interaction of proteins with the implant surface will cause an immune response or hypersensitivity reaction (10). Osteoinduction and bone remodeling are damaged by inflammatory products that affect the implant integration osseointegration resulting in implant failure (7). Biofilm removal includes mechanical debridement, chemical disinfection, antibiotics, and regenerative surgical therapy with therapeutic interventions through its microbiological repair (74). Osteointegration in dental implants through hADMSC seeding will have an impact on reducing dental implant failure.

The selection of biomaterials needs to be developed to prevent allergic responses that lead to implant failure, Y-TZP is the latest biomaterial choice. Sensitivity reactions in titanium biomaterials due to their biocompatible properties, there may be granulomatose reactions and allergies are still debated (48). Low PH conditions and chemical compounds such as cetylpyridium chloride, sodium fluoride and hydrogen peroxide occur in dental implants (46). Allergic reactions can lead to decreased osseointegration due to the presence of peri-implanitis that is expected to be biocompatible dental implant biomaterials (49). Ceramics have the ability to produce a surface layer of apatites, but they have mechanical susceptibility and cannot replace bones (45). The occurrence of osseointegration in bones and soft tissues to prevent bacteria from entering and affect the success of dental implants which have mechanical properties, biological stability and biocompatibility as well as aesthetics (75). Y-TZP has good biocompatibility and aesthetics as a dental restoration (76). The use of Y-TZP biomaterial in tissue engineering can be used in other fields of medicine, due to its good biocompatibility as implant biomaterial and other orthopedic biomaterials.

Infection control is needed to prevent Dental implant failure by administering hADMSC seeded in Y-TZP. The biocompatibility of Y-TZP as a dental implant material is in line with the human transcriptome in two pathways, namely mineral absorption and immune response(77). Evaluation of Y-TZP through evaluation of cell metabolic activity, cytotoxicity and potential inflammation of oral fibroblasts (78). There is an orientation of gingival fibers towards the surface of the Y-TZP implant through the effect of fibronectin immobilization, so that Y-TZP adsorbs atelocollagen through fibronectin preadsorption (79). Enzymatic degradation occurs in Y-TZP (80). Increased plastic deformation and ceramic surface defects are present in Y-TZP (81). Immune response due to infection can be prevented Y-TZP biomaterial seeded with hADMSC has good biocompatibility properties to avoid dental implant failure.

## 4. Conclusion

Dental implant failure can be prevented by hADMSC seeding intervention on Y-TZP biomaterial through Increased Runx-2 expression in dental implant integration. Runx-2 in osteogenesis and fibroblast migration are influenced by various genes in the bone healing process. The benefits of this research can reduce the stiffness of dental implants.

## Conflict of Interest

The authors declare no conflicts of interest.

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